Electron Microscopy of Hemosiderin: Presence of Ferritin and Occurrence of Crystalline Lattices in Hemosiderin Deposits*

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PLATES 21 AND 22

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ABSTRACT

Injections of hemoglobin were given to rats in order to produce hemosiderosis, and selected hemosiderin granules in sectioned cells of proximal convoluted tubules were studied by means of electron microscopy. When examined at high resolution, many of the dense particles that were present in hemosiderin granules proved to have the structure that characterizes the iron hydroxide micelles of molecular ferritin. In some hemosiderin deposits the dense particles formed lattices similar to those present in sections of crystalline ferritin. Such ordered arrangement of dense particles was encountered inside as well as outside of the cytoplasmic organelles for which the name "siderosomes" has been proposed previously, and which may be derived from mitochondria. Study of hemosiderin granules in hepatic parenchymal and reticuloendothelial cells of human beings yielded similar results. The findings confirm the inference that ferritin is a component of hemosiderin, and they indicate that some of the so called hemosiderin granules are crystals of ferritin.

In a previous report (1) the author has described the fine structure and disposition of hemosiderin in different types of cells. From the evidence presented it was inferred (1) that the iron micelles of hemosiderin and ferritin are composed of the same subunits; (2) that ferritin is a component of hemosiderin and at times the predominant one; and (3) that the formation of hemosiderin and ferritin is associated with discrete cytoplasmic organelles for which the name "siderosomes" was proposed, and which seem to be derived from mitochondria.

The findings now to be presented demonstrate that typical quadruplets of iron micelles—characteristic of ferritin—are present in deposits of hemosiderin; and that some hemosiderin granules, inside as well as outside of siderosomes, display lattice structure similar to that present in crystals of ferritin.

Materials and Methods

General.—The procedures were similar to those employed in the previous study (1). To produce hemosiderosis, rats were given repeated intraperitoneal injections of rat hemoglobin solutions. Tissue taken from the kidneys was then studied by means of light and electron microscopy. Similarly, tissue taken from spleen and liver of two patients who are suffering from transfusional hemosiderosis associated with Cooley's anemia was also studied.

Animals.—Five male and five female albino rats of Wistar strain, weighing approximately 150 gm., were used. They were given purina chow and water *ad libitum.*

Preparation and Injection of Hemoglobin.—This was done as reported previously (1), three to five injections being given intraperitoneally at intervals of 2 days.

Material from Human Patients.—A therapeutic splenectomy and a liver biopsy were performed on each of two children (10 and 7 years old, respectively), who are suffering from Cooley's anemia. Both had received well over a hundred transfusions of whole blood.

Preparation of Tissues.—The tissues were processed for electron microscopy and subjected to histochemical tests as described in detail in the previous report (1, 6).

Crystallization and Preparation of Ferritin for Electron Microscopy.—Using a procedure described by Granick (2), ferritin was crystallized from aliquots of spleen from the two patients and from pooled

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livers of hemonderotic rats. Solutions of ferritin were prepared with the purified crystals as described before (1).

To obtain sections of ferritin crystals, the following procedure was employed. A drop taken from a suspension of crystals in distilled water was put on a coverslip. The latter was then inverted over a well-slide into which several drops of 10 per cent formalin has been put. The crystals were thus exposed to formaldehyde vapor for 1 hour. Afterward they were dehydrated in graded concentrations of ethyl alcohol, embedded in *n*-butyl methacrylate, and sectioned in the same manner as the blocks of tissue.

Electron Microscopy.—Sections were mounted on copper specimen grids that had been coated with carbon films. They were then examined with an RCA electron microscope, Model EMU-3b, using a 100 KV beam and a combination of objective and projector apertures to be described elsewhere (3). Figs. 7 and 8 were taken with a Siemens Elmiskop I.

OBSERVATIONS

To learn more about the structure of the dense components of hemosiderin granules, cells in the proximal convoluted tubules of rats given multiple intraperitoneal injections of hemoglobin were studied first. The presence of hemosiderin was ascertained by means of light microscopy as previously described (1). Electron micrographs of typical granules were generally taken at high primary magnifications (above 20,000). Fig. 1 shows aggregated and scattered dense particles in a siderosome situated in a cell of a proximal convoluted tubule. Most of these particles have diameters of about 60 A. As shown in Figs. 2 and 3, enlargements of particles in this and other siderosomes reveal subunits with diameters of about 30 A, often forming "quadruplets" similar to those of molecular ferritin, as first described by Farrant (4), and confirmed by the author (1), and by Kuff and Dalton (5).

It should be borne in mind that in electron micrographs the configuration of the subunits of the dense particles is partly dependent upon their orientation in the specimen with respect to the electron beam. Thus, when viewed sidewise in a two-dimensional projection, a "quadruplet" may appear as a "doublet," or as a particle with poorly defined subunits. Furthermore, owing to the fact that the depth of field provided in our instrument at top magnification is about 70 A, and thus less than the usual thickness of our sections (about 200 A), all of the particles cannot be in focus at the same time.

In further work it was noted that in some of the hemosiderin granules the dense particles were arranged in ordered arrays (Figs. 4 to 6). When compared with the molecular lattice structure of sectioned crystals of ferritin (Figs. 10 and 11), a fundamental similarity becomes evident, for the lattices of the intracellular deposits resemble those of crystalline ferritin in respect to size, arrangement, and subunits of the component dense particles.

Some irregularities in the lattices depicted in Figs. 4 to 6 are evident, but these are undoubtedly artifacts resulting from processing of the tissues, especially from sectioning, as numerous observations on sections of crystalline ferritin have shown (Fig. 10). The images shown in Figs. 1 to 6 do not, of course, give information about the component of hemosiderin that is *not* opaque to electrons, but it can be inferred that this component is distributed between and around the dense particles. Moreover, the dense particles could hardly display ordered arrangements as shown in Figs. 4 to 6 if the non-dense component associated with them were not in appropriate order. Further comment on this problem will be given in the Discussion.

To learn whether the observations just described have a more general validity, hemosiderin granules in parenchymal liver cells and in splenic macrophages and sinusoidal endothelial cells from two patients who are suffering from transfusional hemosiderosis were also examined. Close scrutiny of many hemosiderin deposits in both liver and spleen, has confirmed the observations made on rat kidney cells. For example in Figs. 7 and 8, detail can be seen in particles within and about hemosiderin granules, often in the form of four subunits with diameters of approximately 30 A. Again, some of the hemosiderin granules displayed lattices (Fig. 9) similar to those in crystalline human ferritin (Figs. 10 and 11), and similar to those found in the experimental rats (Figs. 5 and 6).

DISCUSSION

There are two salient points in the evidence just given. Briefly stated, these are: (1) that many of the dense particles in hemosiderin have the structure that characterizes the iron micelles of molecular ferritin, and (2) that some hemosiderin granules, inside as well as outside of siderosomes, exhibit lattices similar to those demonstrable in crystalline ferritin.

The reasons for the inference that the electron dense particles in hemosiderin are composed of iron hydroxide micelles have been given in an earlier report. However, in the earlier work the subunits that characterize the dense component of ferritin molecules were demonstrated only in material isolated from hemosiderotic tissues, and not in sectioned cells. The inference that molecular ferritin was present in the intact cells was based partly on size-frequency distributions of the particles in electron micrographs of sectioned cells, and partly on other considerations.

It has already been mentioned that the iron micelles of ferritin molecules occur mostly as "quadruplets" that have a characteristic orientation and are situated within the apoferritin (4). It might be said that the demonstration provided in the present report relates only to iron micelles, and that their presence does not of itself establish the presence of the protein component of ferritin, the apoferritin. There are, however, reasons why it is likely that the majority of the "quadruplets" in hemosiderin are associated with apoferritin. In the first place, ferritin can be extracted from hemosiderotic tissues in direct proportion to the amount of hemosiderin present (13). In our hands, preparations of such ferritin, shadowed with chromium, have given images identical with those discovered by Farrant (4). Secondly, Kuff and Dalton (5) have isolated dense particles from homogenates of normal liver cells by means of ultracentrifugation which, when shadowed, had the characteristic appearance of ferritin molecules. Thirdly, there is the finding of ordered intracellular aggregates of dense particles which correspond in size, structure, and arrangement to the cubic lattices demonstrable in crystals of ferritin. Such aggregates, which have the essential attributes of crystals, could scarcely have been formed if their component molecules were not relatively homogeneous. Since iron constitutes at most only about 35 per cent of the dry mass of hemosiderin (7), it does not seem likely that crystallization would occur if the remainder were heterogeneous. If, on the other hand, the non-dense component were relatively homogeneous, then it would be reasonable to suppose that it consists of apoferritin for such a supposition is in harmony with the findings as a whole and with the work done by others (2, 8, 9). It is hoped that the matter may be settled by means of x-ray diffraction of suitably prepared material.

The observation that "crystalline" bodies of hemosiderin can be present within siderosomes as well as outside of them is open to different interpretations. In view of the disruptive changes that take place in siderosomes (1), it seems likely that the crystals are formed within siderosomes and subsequently liberated.

It is to be emphasized, however, that only a small proportion of hemosiderin granules showed crystalline order. This is in accord with the histochemical findings of Gedigk and Strauss (10, 11), and of Goessner (12), which indicate that most hemosiderin granules have a relatively heterogeneous composition, particularly in respect to their organic components. Such granules should not display crystalline order. Using the histochemical methods employed by these workers, one cannot, however, distinguish between granules situated within siderosomes and those free of siderosomes. Histochemical tests for organic components of hemosiderin as used by these authors might give falsely positive results if parts of the siderosomes other than the enclosed hemosiderin should react. Moreover, it cannot be justifiably inferred from their evidence that *all* hemosiderin granules are heterogeneous, for the methods employed were highly selective. It should be added here that Shoden, Gabrio, and Finch (13) have studied the relationship between ferritin and hemosiderin with the aid of tissue fractionation procedures and of radioiron. They have concluded that the two compounds "are intimately associated and are functionally indistinguishable," and that "it appears likely that these two compounds differ only in physical form."

Addendum.—After this paper had gone to press, a report by M. Bessis and J. Breton-Gorius was published (*Compt. rend. Acad. Sc.*, **245**, 1271) in which aggregates of typical "quadruplets" of iron micelles were demonstrated in erythroblasts and reticuloendothelial cells. These authors also found crystalline lattice structure in some hemosiderin deposits in liver cells. The findings are in agreement with those presented in this paper and in a previous report (1), and provide strong evidence for the view that iron depots in different types of cells are mainly present in the form of ferritin.

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Explanation of Plates

Plate 21

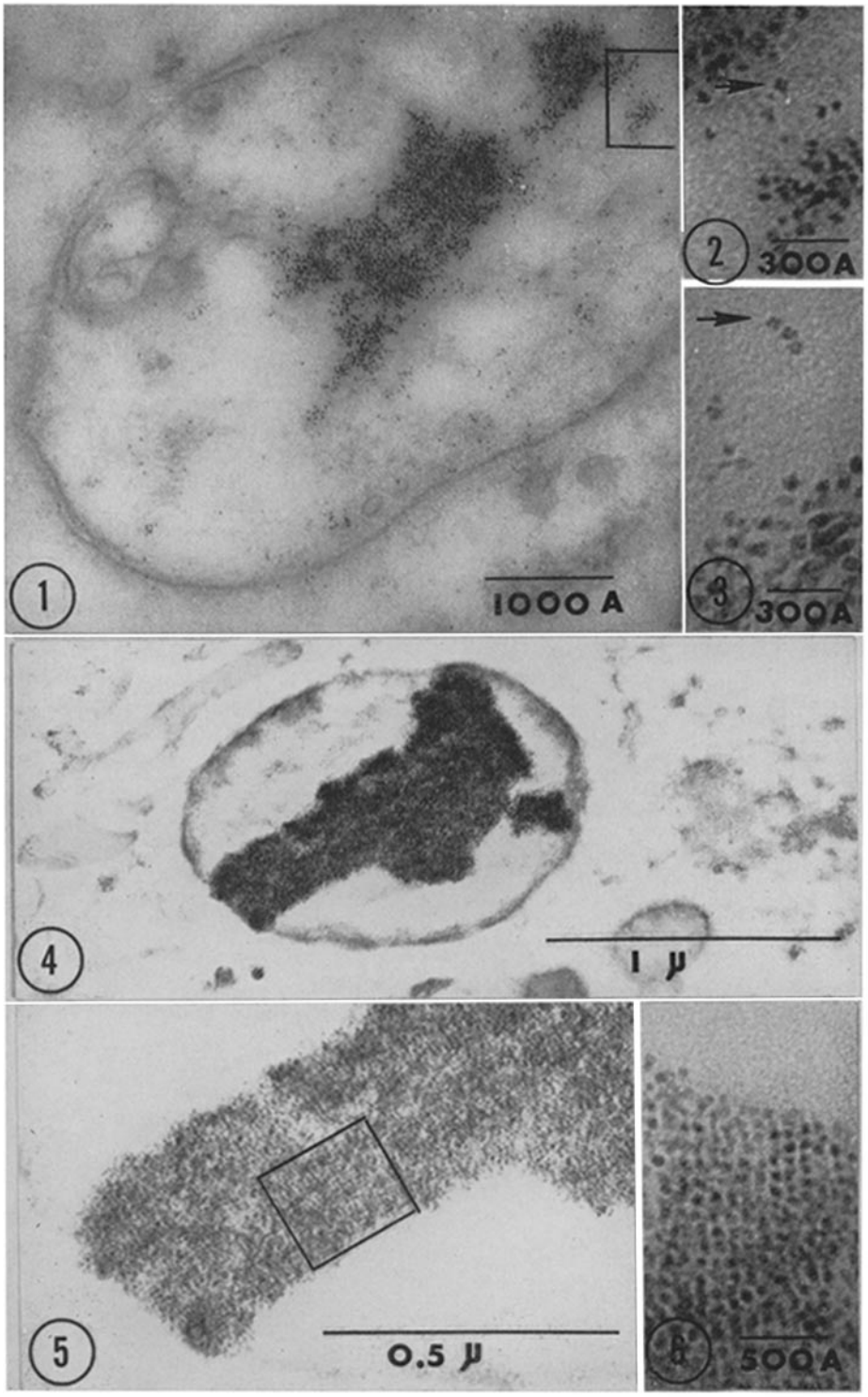
Fig. 1. Siderosome in cell of proximal convoluted tubule from rat treated with hemoglobin. The aggregated dense particles represent iron micelles of a hemosiderin deposit and have a mean diameter of about 60 A. × 83,000.

Fig. 2. Enlargement of area delineated in Fig. 1. The arrow points to an electron dense particle that is composed of four smaller "subunits." The "quadruplet" as a whole has a diameter of about 60 A while the subunits measure 30 A in diameter. The quadruplet has the size and appearance that characterize the iron micelles of ferritin as seen in electron micrographs. × 333,000.

Fig. 3. Part of another hemosiderin deposit in cell of proximal convoluted tubule from the same kidney. The arrow points to a "quadruplet." Next to this is a "triplet." Some of the particles in the lower half of the picture also display internal structure. × 333,000.

Figs. 4 and 5. Siderosome in cell of proximal convoluted tubule from another rat that had received injections of hemoglobin. The closely packed aggregate of dense particles within the siderosome represents a hemosiderin granule large enough to be detectable with a light microscope. Greater enlargement (Fig. 5) reveals organization that most hemosiderin granules do not show. In areas, delineated in Fig. 5, the dense particles appear to be in lattice order. Fig. 4, × 42,000; Fig. 5, × 84,000.

Fig. 6. Part of another granule in the same section. Ordered arrangement of particles in two dimensions at right angles is evident in spite of distortions produced by sectioning. Note also that some of the particles appear to have internal structure. × 250,000.



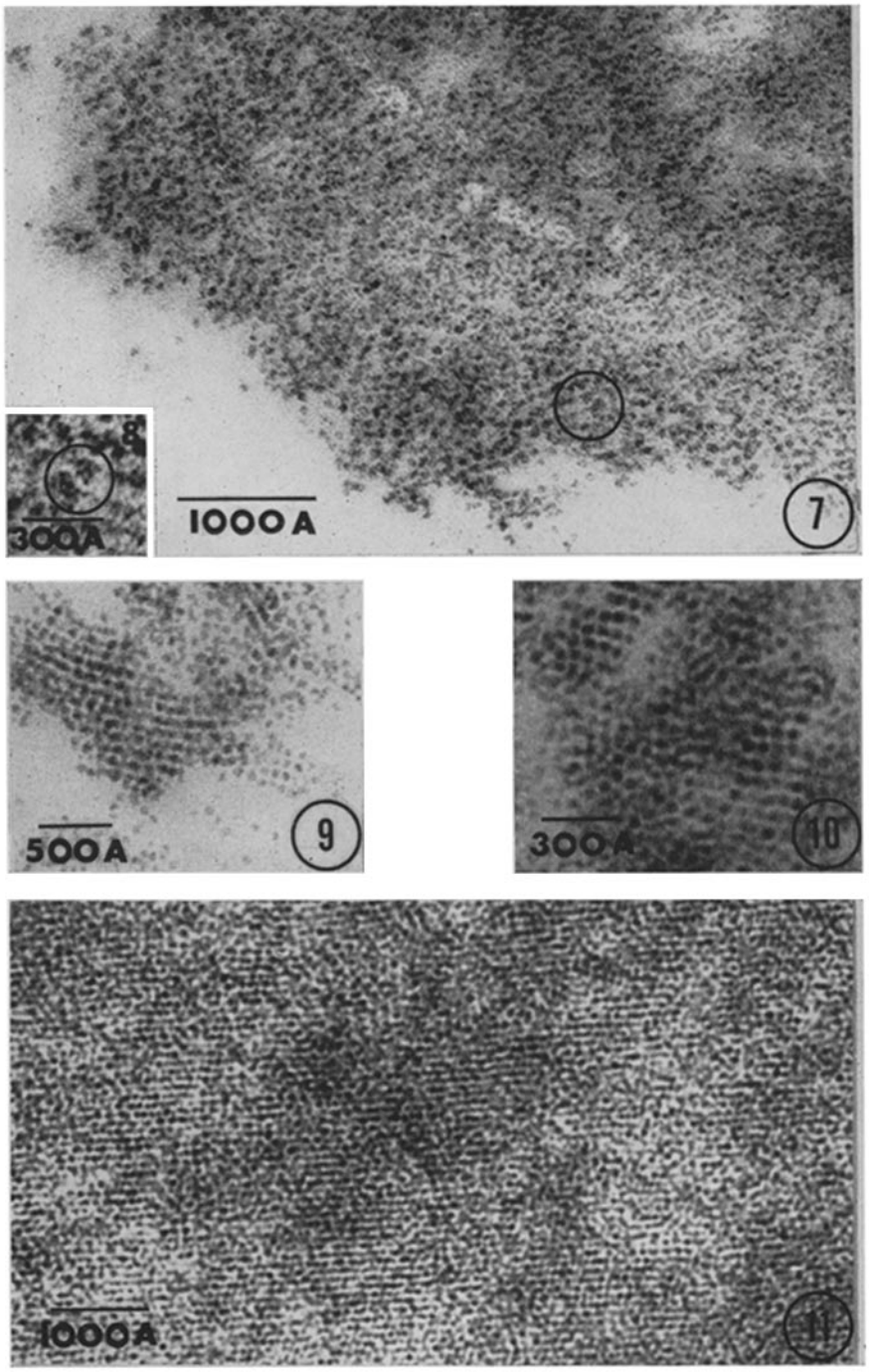
(Richter: Electron microscopy of hemosiderin)

PLATE 22

FIGS. 7 and 8. Part of hemosiderin granule in liver cell of biopsy specimen from a patient who has transfusional hemosiderosis. Most of the dense particles show internal structure. Fig. 8 is an enlargement of the area circled in Fig. 7, and shows two quadruplets of 30 A units that have the appearance of iron micelles of ferritin. Fig. 7, × 182,000; Fig. 8, × 364,000.

FIG. 9. Part of hemosiderin deposit in another liver cell of the same biopsy specimen. Compare with Fig. 6. × 200,000.

FIGS. 10 and 11. Electron micrographs of sectioned crystals of ferritin prepared from spleen of patient who has hemosiderosis. To obtain the image shown in Fig. 11 a relatively thick section (about 700 A) was used. The image in Fig. 10 was made with a thinner section (about 200 A). Note the better preservation of crystalline lattice in Fig. 11, but the somewhat better resolution in Fig. 10. It is inferred from pictures such as these that the imperfections of lattices shown in Figs. 5, 6, and 9 are partly the result of sectioning. Fig. 10, × 333,000; Fig. 11, × 133,000.



(Richter: Electron microscopy of hemosiderin)